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*rbc*L 基因序列分析对连香树科和交让木科系统位置的重新评价——兼论低等金缕梅类的关系[*]

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A REVALUATION OF THE SYSTEMATIC POSITIONS OF THE CERCIDIPHYLLACEAE AND DAPHNIPHYLLACEAE BASED ON *rbc*L GENE SEQUENCE ANALYSIS, WITH REFERENCE TO THE RELATIONSHIP IN THE "LOWER" HAMAMELIDAE

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Abstract The *rbc*L gene sequences of six species representing five subfamilies of the Hamamelidaceae and the Platanaceae were determined and used in the phylogenetic analysis on the "lower" Hamamelidae sensu Endress (1989) and its allies newly suggested. Four most parsimonious trees were obtained, all having 893 steps with CI = 0.558 and RI = 0.591. The families Cercidiphyllaceae, Daphniphyllaceae, Hamamelidaceae and Saxifragaceae are closely located, while the relationships among them remain unsolved even if more representatives of the Hamamelidaceae were further added in this parsimony analysis. Our results confirm the phylogenetic trees revealed by Chase *et al*. (1993) and Soltis *et al*. (1997), instead of those of Hoot and Crane (1996). Considering the morphological features they share, it is suggested that the Cercidiphyllaceae and Daphniphyllaceae be placed into the Hamamelidales. The relationship between the Platanaceae and the Hamamelidaceae shown in our analysis is not so closed as suggested by the cladistic analyses by using morphological characters only(e.g. Lu *et al*., 1991), while those among the Platanaceae, Trochodendraceae and Tetracentraceae are close as indicated by this study. The Eupteleaceae falls into the Ranunculales. The Eucommiaceae seems to show closer relationship with the Hamamelidaceae, the "core" family of the "lower" Hamamelidae, than with the other members except the Cercidiphyllaceae. The *rbc*L gene trees imply that the "lower" Hamamelidae is a heterogeneous group, composed of isolated ancient families.

Key words The "lower" Hamamelidae; Cercidiphyllaceae; Daphniphyllaceae; Molecular phylogeny; *rbc*L gene

The Hamamelidae, ever since being divided into the "higher" and the "lower" Hamamelidae by some authors, has long been at one of the centres of controversies on the origin and evolution of angiosperms(Crane, 1989). The "lower" Hamamelidae sensu Endress

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(1989) and Hufford and Crane (1989) contains seven families, i.e., the Eupteleaceae, Trochodendraceae, Tetracentraceae, Cercidiphyllaceae, Myrothamnaceae, Platanaceae and Hamamelidaceae. The Cercidiphyllaceae and Daphniphyllaceae, two eastern Asiatic monogeneric families, have been controversial in systematic position, although numerous morphological, palynological, cytological and phytochemical studies have been conducted on them (Endress, 1993, 1989, 1986; Wang & Gao, 1990; Chen, 1989; Morawetz & Samuel, 1989; Sutton, 1989; Zhang & Lu, 1989; Behnke, 1989, 1982, 1980; Heel, 1987; Crane & Stockey, 1986; Zavada & Dilcher, 1986; Bhatnagar & Kapil, 1982; Bogle, 1980; Spongberg, 1979; Bhatnagar & Garg, 1977; Praglowski, 1974; Haruki *et al.*, 1972; Huang, 1966, 1965; Swamy & Bailey, 1949; Croizat, 1941; Harms, 1916). The Cercidiphyllaceae, containing two species, is placed in the Trochodendrales by Dahlgren (1980) and Endress (1986), or in the Hamamelidales by Cronquist (1981) and Thorne(1989, 1983), or as a separate order, Cercidiphyllales, in the Hamamelidae by Takhtajan (1996, 1969). The Daphniphyllaceae is a small family with the number of species ranging from nine to about 40 (Sutton, 1989), but the systematic treatment of this family is very confused. It has even been treated as a member of: (1) the Euphorbiales (Takhtajan, 1969), (2) the Pittosporales (Thorne, 1989, 1983; Croizat, 1941), (3) Buxales (Dahlgren, 1983), (4) the Hamamelidales (Takhtajan, 1980; Hutchinson, 1973); or as a separate order, the Daphniphyllales, near the Euphorbiales (Hurusawa, 1954), the Hamamelidales (Cronquist, 1983; Huang, 1965) or Balanopales (Sutton, 1989).

The unambiguous alignment and slow evolutionary rate of the *rbc*L gene make it appropriate for the investigation on systematics at higher taxonomic hierarchy of angiosperms (Clegg, 1993). Recently, the *rbc*L gene has been used to estimate the phylogeny of the Betulaceae (Bousquet *et al.*, 1992) and the "higher" Hamamelidae (Manos & Steele, 1997) and proved to be very efficient. The analyses of a large range of angiosperm families based on *rbc*L sequences (Chase *et al.*, 1993) and rDNA 18S sequences (Soltis *et al.*, 1997) have revealed the polyphyly of subclass Hamamelidae sensu Cronquist (1981). However, a more focused study is needed in order to yield more convincible conclusions. The purpose of this study is to meet this need and shed some light on the systematic position of the Cercidiphyllaceae and Daphniphyllaceae using *rbc*L gene sequences analysis with more extensive sampling of related taxa.

1 Materials and methods

1.1 Plant materials and DNA isolation

The origin of plant materials was shown in Table 1. Some other taxa with their *rbc*L gene sequences deposited in GenBank (Table 1.) were also used in the present study.

Total genomic DNAs of six species (Table 1.) were isolated from silica-gel dried leaves with a modified CTAB method of Bousquet *et al.* (1990).

1.2 Primer designing

Primers (Table 2.)used in amplifying fragments of *rbc*L and the entire *rbc*L were designed according to the sequence of the *rbc*L gene of *Liquidambar styraciflua* and other hamamelidaceous plants with exception of primer S2R (Hoot *et al.*, 1995).

1.3 DNA amplification and sequencing

The entire *rbc*L gene was amplified by 1F-1496R. In order to obtain the 5′ end sequence of *rbc*L and better sequencing results, the *rbc*L fragments were also amplified by the following primer pairs, i.e., S2R - 414R, 1F - 991R, 1F - 1313R, and 1186F - 1496R.

Table 1　Species analyzed in present research

Order	Family	Species	Voucher/Source	GenBank accession number	Reference
Hamamelidales	Cercidiphyllaceae	*Cercidiphyllum japonicum*	Olmstead 90-016, COLO	L11673	Olmstead *et al*., 1992
Daphniphyllales	Daphniphyllaceae	*Daphniphyllum* sp.	Qiu 91026, NCU	L01901	Albert *et al*., 1992
Hamamelidales	Hamamelidaceae	*Disanthus cercidifolius* ssp. *longipes*	Y. X. Feng 971001 (PE), Hunan	AF081069	the present research
		Rhodoleia henryi	Y. X. Feng 97046 (PE), Kunming	AF081072	the present research
		Exbucklandia populnea	Y. X. Feng 97042 (PE), Kunming	AF081071	the present research
		Mytilaria laosensis	A. M. Lu s. n. (PE), Guilin	AF081070	the present research
		Fortunearia sinensis	D. Y. Hong *et al*. H97018(PE), Henan	AF081068	the present research
		Liquidambar styraciflua	unknown	M58394	Bousquet *et al*., 1992
		Hamamelis mollis	Qiu 91035, NCU	L01922	Albert *et al*.,1992
Hamamelidales	Platanaceae	*Platanus occidentalis*	Y. X. Feng 971101 (PE), Henan	AF081073	the present research
Trochodendrales	Trochodendraceae	*Trochodendron aralioides*	Qiu 91049, NCU	L01958	Albert *et al*., 1992
	Tetracentraceae	*Tetracentron sinense*	Qiu 90009, NCU	L12668	Qiu *et al*., 1993
	Eupteleaceae	*Euptelea polyandra*	Qiu 90026, NCU	L12645	Qiu *et al*., 1993
	Eucommiaceae	*Eucommia ulmoides*	Qiu 91024, NCU	L01917	Albert *et al*., 1992
Saxifragales	Saxifragaceae	*Boykinia rotundifolia*	Gornell 101, UBC	L11175	Morgan & Soltis,1993
		Lepuropetalon spathulatum	Thomas s. n., NLU	L11192	Morgan & Soltis,1993
		Heuchera micrantha	Soltis & Soltis 1949, WS	L01925	Soltis *et al*.,1990
Fagales	Fagaceae	*Quercus rubra*	unknown	M58391	Bousquet *et al*., 1992
	Betulaceae	*Betula papyrifera*	unknown	X56617	Bousquet *et al*., 1992
Juglandales	Myricaceae	*Myrica gale*	unknown	X69530	Maggia & Bousquet, 1994
		Comptonia peregrina	unknown	X69529	Maggia & Bousquet, 1994
Pittosporales	Pittosporaceae	*Hymenosporum flavum*	unknown	U50260	Plunkett *et al*., 1996
Hydrangeales	Hydrangeaceae	*Hydrangea macrophylla*	Morgan 2150, WS	L11187	Morgan & Soltis,1993
Ranunculales	Berberidaceae	*Nandina domestica*	Y.-D. Kim 94-1, TEX	L75871	Kim & Jansen, 1996
	Papaveraceae	*Hypecoum domestica*	unknown	U86628	Hoot *et al*., 1997
Ranunculales	Lardizabalaceae	*Akebia quinata*	Qiu 91020, NCU	L12627	Qiu *et al*., 1993
Magnoliales	Illiciaceae	*Illicium parviflorum*	Qiu 83, NCU	L12651	Qiu *et al*., 1993
Magnoliales	Winteraceae	*Drimys winteri*	Qiu 90016, NCU	L01905	Albert *et al*., 1992
Magnoliales	Magnoliaceae	*Magnolia hypoliuca*	Qiu 24, NCU	L12655	Qiu *et al*., 1993
		Manglieta fordiana	Qiu 26, NCU	Li2658	Qiu *et al*., 1993

Table 2 Primer sequences and locations. The base positions of the primers are determined according to the *rbc*L gene sequence of *Liquidambar styraciflua*.

Primer	Sequence (5′~3′)	Location
S2R	GAAGTAGTAGGATTGATTCTC	*atp*B: 2R (Hoot *et al*., 1995)
1F	ATGTCACCACAAACAGAAACT	*rbc*L: 1-21
414R	CAAATCCTCCAGACGTAGAGC	*rbc*L: 414-394
991R	CGGTACCAGCGTGAATATGAT	*rbc*L: 991-971
1186F	CTGACCGAGATCTTTGGGG	*rbc*L: 1168-1186
1313R	ATTTCATTACCTTCACGAGC	*rbc*L: 1332-1313
1496R	GATTGGGCCGAGTTTAATTAC	*rbc*L: 1496-1474

After the double-strand PCR products were purified with Wizard DNA Clean-up System (Promega, Ltd), they were directly sequenced using Silver Sequence™ DNA Sequencing System (Promega, Ltd.) following detail protocols of Wang & Li (1998).

1.4 Sequence alignment and phylogenetic analysis

The sequences were aligned with DNASIS software, and base positions from 1 to 1428 of the *rbc*L were used in the present study.

Since the systematic position of the "lower" Hamamelidae has oscillated between magnolialian and hamamelidalian affinities(Endress, 1986), and its some members have been related to ranunculids, two outgroups, viz. *Magnolia hypoleuca* and *Manglieta fordiana* (Magnoliaceae), were selected. Although *Eucommia ulmoides* (Eucommiaceae) is not placed in the "lower" Hamamelidae in the modern systems of angiosperms, it was included in the present analysis because its close relationship with *Cercidiphyllum* has been suggested (cf. Hufford & Crane, 1989). Some species of the "higher" Hamamelidae (e.g. *Betula papyrifera*, *Quercus rubra*, *Comptonia peregrina*, *Myrica gale*) and *Hymenosporum flavum* (Pittosporaceae) were analyzed together because close relationships to the Daphniphyllaceae were suggested by previous studies(Crane, 1989; Thorne, 1983).

The aligned sequences were analyzed with the PAUP 3.1.1 computer program (Swofford, 1993) to search for the shortest trees through heuristic search, with simple sequence addition, "TBR" branch swapping, MULPARS and Steepest descent options in effect, automatic increase by 100 and collapse of Zero-length branches. Bootstrapping of 100 replications was conducted to assign clade support, bootstrap 50% majority-rule consensus tree was obtained with the same options as those used in finding the most parsimonious trees.

2 Results

Four equally most parsimonious(MP) trees with 893 steps are obtained, and the CI, RI and RC values of the trees are 0.558, 0.591 and 0.330, respectively. *Drimys winteri* is first separated from the other ingroups. The next clade is *Illicum*, followed by a strongly supported clade(100% BP), which comprises *Euptelea* (a member of the "lower" Hamamelidae) and three members of ranunculids. Three members of the "lower" Hamamelidae (Platanaceae, Trochodendraceae and Tetracentraceae) form a clade which is separated from the Hamamelidaceae-Saxifragaceae(s.l.)-the "higher" Hamamelidae clade by the *Eucommia*-*Hymenosporum*-*Hydrangea* clade. The Daphniphyllaceae and Cercidiphyllaceae nest within the weakly supported clade composed of the Hamamelidaceae(s. l.), the Saxifragaceae(s. l.) and the "higher" Hamamelidae. The relationships among the subfamilies of the Hamamelidaceae are not resolved. One of the most parsimonious trees (Fig. 1) is used to indicate branch lengths and bootstrap values. The strict consensus tree of 4 most parsimonious trees is shown in Fig. 2. Pairwise genetic divergence values are presented in Table 3.

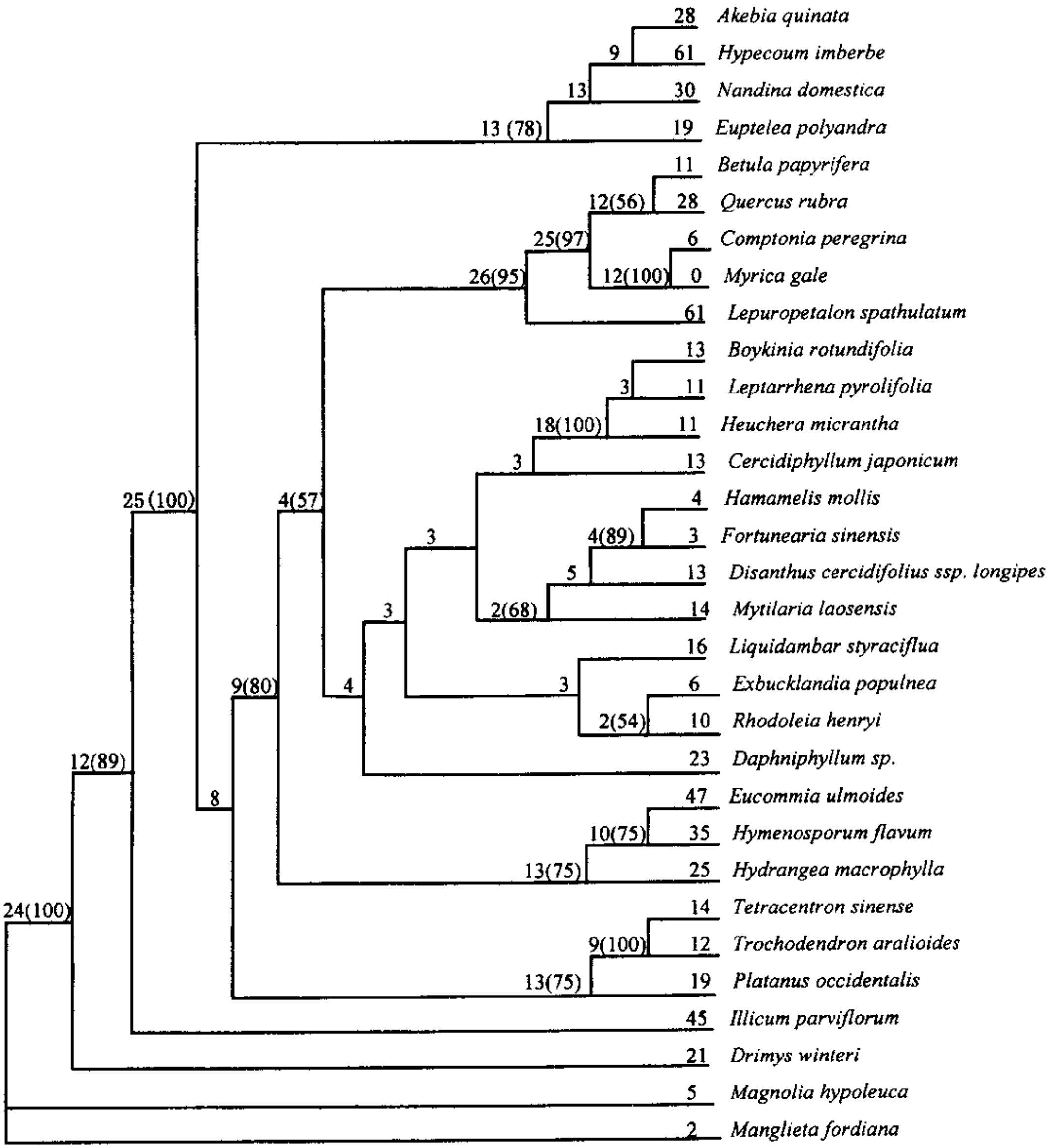

Fig. 1　One of four equally most parsimonious trees resulted from phylogenetic analysis of *rbc*L gene sequence data. The tree has a length of 893 steps, CI = 0558, and RI = 0591. The branch lengths are given above the branches and bootstrap values above 50 are shown in parentheses

3　Discussion

Recently, the Cercidiphyllaceae is placed (1) in the Hamamelidales (Spicliger & Savolainen, 1997; Thorne, 1983; Cronquist, 1981), (2) in the Hamamelidae as a separate

Table 3 Pairwise genetic distances of *rbc*L gene sequences

OUTs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	–	0.071	0.058	0.069	0.051	0.052	0.067	0.072	0.041	0.050	0.061	0.061	0.056	0.065	0.077
2	102	–	0.057	0.027	0.053	0.057	0.079	0.069	0.068	0.047	0.054	0.060	0.058	0.080	0.088
3	83	81	–	0.055	0.029	0.039	0.064	0.060	0.050	0.029	0.018	0.053	0.049	0.068	0.075
4	98	39	79	–	0.052	0.055	0.074	0.075	0.064	0.050	0.053	0.062	0.061	0.078	0.089
5	71	74	41	73	–	0.026	0.064	0.061	0.048	0.020	0.029	0.047	0.038	0.068	0.072
6	74	82	55	79	37	–	0.060	0.061	0.047	0.025	0.039	0.048	0.039	0.073	0.069
7	95	113	91	105	90	86	–	0.082	0.055	0.062	0.064	0.068	0.067	0.073	0.055
8	103	98	86	107	86	87	117	–	0.069	0.055	0.062	0.057	0.056	0.083	0.086
9	59	97	72	92	67	67	79	98	–	0.046	0.055	0.054	0.052	0.061	0.065
10	71	67	42	71	28	35	89	78	65	–	0.027	0.046	0.036	0.068	0.068
11	87	77	26	75	41	55	91	88	78	38	–	0.053	0.047	0.071	0.077
12	87	86	75	89	66	68	97	82	77	66	75	–	0.048	0.079	0.083
13	80	83	70	87	53	56	95	80	74	52	67	68	–	0.073	0.073
14	89	109	92	106	92	100	100	113	83	92	97	107	100	–	0.087
15	110	125	107	127	101	98	78	123	93	97	110	118	104	119	–
16	85	77	24	77	43	56	89	86	77	37	24	80	68	96	107
17	123	100	109	97	104	107	131	120	111	94	111	114	112	129	133
18	79	68	48	75	34	40	93	85	71	26	47	77	58	99	96
19	89	103	89	96	78	79	50	117	74	80	87	92	89	107	74
20	89	104	92	97	80	74	47	118	71	77	90	91	88	109	75
21	99	33	80	6	74	80	104	104	90	72	74	88	86	105	127
22	65	102	85	100	78	76	92	105	61	74	87	82	89	94	115
23	109	39	93	58	83	92	122	105	107	76	90	96	96	120	131
24	75	74	46	78	34	45	92	85	70	21	44	76	62	95	95
25	71	63	39	66	25	36	86	72	65	21	37	68	53	89	95
26	70	66	42	73	25	35	91	76	66	7	39	69	51	94	95
27	77	72	45	74	30	44	90	84	71	26	44	71	56	96	100
28	68	64	38	68	27	38	85	76	64	23	39	67	56	87	92
29	76	89	72	87	57	61	91	86	65	53	67	78	66	93	97
30	81	93	72	89	59	58	92	88	66	57	68	78	73	95	101
31	61	78	60	78	48	48	68	76	48	45	57	62	60	69	77

Note: Below diagonal are absolute distances while above diagonal are mean distances. OUTs: 1. *Akebia quinata*; 2. *Betula papyrifera*; 3. *Boykinia rotundifolia*; 4. *Comptonia peregrina*; 5. *Cercidiphyllum japonicum*; 6. *Daphniphyllum sp.*; 7. *Drimys winteri*; 8. *Eucommia ulmoides*; 9. *Euptelea polyandra*; 10. *Hamamelis mollis*; 11. *Heuchera micrantha*; 12. *Hymenosporum flavum*; 13. *Hydrangea macrophylla*; 14. *Hypecoum domestica*; 15. *Illicum parviflorum*;

order, the Cercidiphyllales (Takhtajan, 1996, 1969) and (3) in the Trochodendrales (Endress, 1986; Dahlgren 1980). Hallier placed "*Cercidiphyllum* as a subfamily Cercidiphylleae before the Bucklandioideae" in 1903 (cf. Tang, 1943). Hufford & Crane (1989) presents a cladistic analysis of the "lower" Hamamelidae, which showes that: (1) the Hamamelidaceae is monophyletic and has a sister group relationship with *Platanus*; (2) *Cercidiphyllum*, *Myrothamnus*, *Euptelea*, and *Eucommia* are the sister groups of *Platanus* and Hamamelidaceae. Another cladistic analysis of the basal Hamamelidae with 92 characters (Schwarzwalder & Dilcher, 1991) supports a close phylogenetic relationship between the subfamily Altingioideae (Hamamelidaceae) and the Platanaceae . In a parsimony of 31 species related to the "lower" Hamamelidae.

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
0.060	0.086	0.055	0.062	0.062	0.069	0.046	0.076	0.053	0.050	0.049	0.054	0.048	0.053	0.057	0.043
0.054	0.070	0.048	0.072	0.073	0.023	0.071	0.027	0.052	0.044	0.046	0.050	0.045	0.062	0.065	0.055
0.017	0.076	0.034	0.062	0.064	0.056	0.060	0.065	0.032	0.027	0.029	0.032	0.027	0.050	0.050	0.042
0.054	0.068	0.053	0.067	0.068	0.004	0.070	0.041	0.055	0.046	0.051	0.052	0.048	0.061	0.062	0.055
0.031	0.074	0.024	0.056	0.057	0.053	0.056	0.059	0.024	0.018	0.018	0.021	0.019	0.041	0.042	0.034
0.039	0.075	0.028	0.055	0.052	0.056	0.053	0.064	0.032	0.025	0.025	0.031	0.027	0.043	0.041	0.034
0.062	0.092	0.065	0.035	0.033	0.073	0.064	0.085	0.065	0.060	0.064	0.063	0.060	0.064	0.064	0.048
0.060	0.084	0.060	0.082	0.083	0.073	0.074	0.074	0.060	0.050	0.053	0.059	0.054	0.060	0.062	0.053
0.054	0.078	0.050	0.052	0.050	0.063	0.043	0.075	0.049	0.046	0.046	0.050	0.045	0.046	0.046	0.034
0.026	0.066	0.018	0.056	0.054	0.050	0.052	0.053	0.015	0.015	0.005	0.018	0.016	0.037	0.040	0.032
0.017	0.078	0.033	0.061	0.063	0.052	0.061	0.063	0.031	0.026	0.027	0.031	0.028	0.047	0.048	0.040
0.056	0.080	0.054	0.064	0.064	0.062	0.057	0.067	0.054	0.048	0.048	0.050	0.047	0.055	0.055	0.043
0.048	0.078	0.041	0.062	0.062	0.060	0.062	0.067	0.044	0.037	0.036	0.039	0.040	0.046	0.051	0.042
0.070	0.095	0.073	0.079	0.080	0.077	0.069	0.088	0.070	0.065	0.069	0.070	0.064	0.068	0.070	0.051
0.075	0.093	0.067	0.052	0.053	0.089	0.081	0.092	0.067	0.067	0.067	0.070	0.065	0.068	0.071	0.054
–	0.071	0.030	0.062	0.062	0.055	0.059	0.063	0.029	0.026	0.027	0.032	0.028	0.045	0.046	0.039
102	–	0.069	0.092	0.090	0.067	0.076	0.074	0.070	0.062	0.066	0.067	0.064	0.079	0.081	0.061
43	99	–	0.059	0.057	0.051	0.059	0.057	0.022	0.017	0.016	0.025	0.020	0.040	0.043	0.036
88	131	84	–	0.005	0.067	0.066	0.082	0.061	0.054	0.055	0.058	0.056	0.059	0.060	0.046
89	128	82	7	–	0.067	0.065	0.083	0.064	0.056	0.055	0.059	0.058	0.060	0.061	0.046
78	96	73	95	96	–	0.071	0.036	0.055	0.047	0.051	0.053	0.049	0.062	0.063	0.055
84	109	84	94	93	101	–	0.081	0.057	0.052	0.053	0.054	0.051	0.062	0.062	0.048
90	105	81	117	118	52	115	–	0.060	0.052	0.052	0.059	0.054	0.068	0.071	0.061
41	99	31	87	90	78	81	85	–	0.018	0.014	0.023	0.019	0.042	0.044	0.034
37	89	24	77	80	67	74	74	25	–	0.013	0.018	0.011	0.037	0.037	0.027
38	94	23	79	78	73	75	74	20	19	–	0.018	0.015	0.036	0.040	0.032
46	96	36	83	84	75	77	84	32	25	25	–	0.020	0.046	0.047	0.036
39	90	28	79	82	69	72	76	27	16	21	29	–	0.037	0.037	0.030
64	113	57	84	85	88	89	97	59	53	52	66	53	–	0.018	0.027
65	115	62	86	87	90	88	101	62	53	57	67	53	26	–	0.028
56	87	51	66	66	79	68	87	48	38	46	51	42	39	40	–

16. *Leptarrhena pyrolifolia*; 17. *Lepuroperalon spathulatum*; 18. *Liquidambar styraciflua*; 19. *Magnolia hypoleuca*; 20. *Manglietia fordiana*; 21. *Myrica gale*; 22. *Nandina domestica*; 23. *Quercus rubra*; 24. *Disanthus cercidifolius* ssp. *longipes*; 25. *Exbucklandia populnea*; 26. *Fortunearia sinensis*; 27. *Mytilaria laosensis*; 28. *Rhodoleia henryi*; 29. *Tetracentron sinense*; 30. *Trochodendron aralioides*; 31. *Platanus occidentalis*.

analysis of 49 taxa of Magnoliidae, Hamamelidae and Alismatidae, with gymnosperms as the outgroup for the polarization of 104 characters, Loconte & Stevenson(1991) also constructed a phylogeny of the "lower" Hamamelidae, in which the Eupteleaceae is the first basal group of Hamamelidae followed by two clades, i.e., the Platanaceae-Hamamelidaceae clade and the Cercidiphyllaceae-Tetracentraceae-Trochodendraceae clade. At the same time, Lu *et al*. (1991) made similar conclusion on phylogeny of the "lower" Hamamelidae through a cladistic analysis of 19 families of the subclass Hamamelidae with 32 characters. According to them, the Eupteleaceae, Platanaceae and Myrothamnaceae form a clade close to the Hamamelidaceae , while the Trochodendraceae - Tetracentraceae clade is first separated from

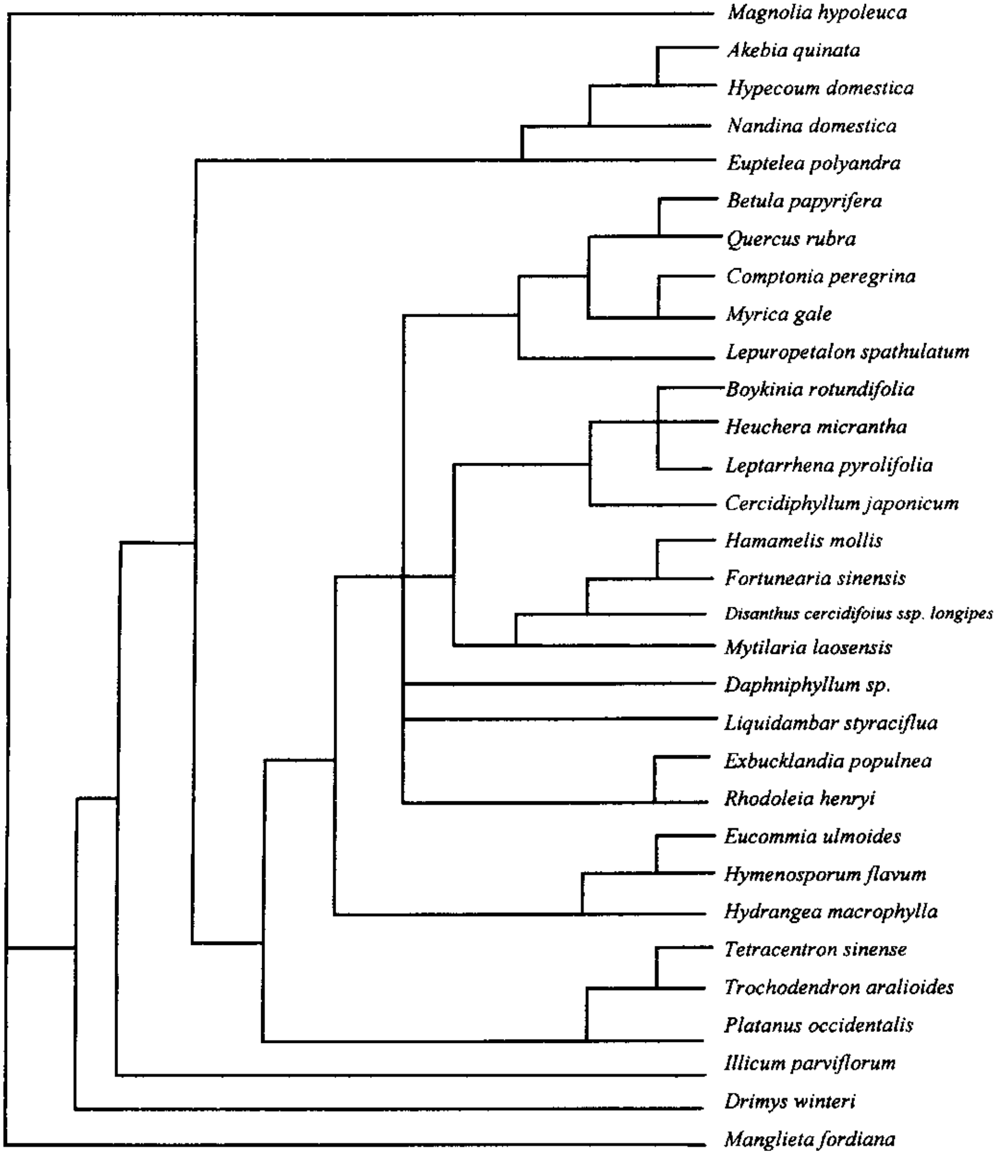

Fig. 2 The strict consensus tree of the four most parsimonious trees.

the other members of the subclass Hamamelidae. Although these results differ from each other inmany respects, they are common in that the Platanaceae is a sister group of the Hamamelidaceae, while the Cercidiphyllaceae is not closely related to the Hamamelidaceae.

Constructing the phylogeny of the "lower" Hamamelidae is very difficult due to numerous extinction events and reduced reproductive organs. The molecular data of low homoplasy level are usually proved to be very efficient in inferring the phylogeny of higher taxonomic rank and the *rbc*L gene sequence data, in particular, have shown great importance in constructing phylogeny of seed plants. The largest analysis of *rbc*L sequence (Chase *et al.*, 1993) suggests the polyphyly of the "lower" Hamamelidae and shows that both the Cercidiphyllaceae and Daphniphyllaceae nest with the Hamamelidaceae and Saxifragaceae, but probably due to poor representative of the related taxa, a detail discussion is not presented. Soltis *et al*. (1997) obtained the similar results by means of 18S rDNA sequence analysis. The polyphyly of the "lower" Hamamelidae is revealed again, and the Cercidiphyllaceae and Daphniphyllaceae are located in the Saxifragoids clade with their neighbor, Hamamelidaceae. However, representatives of the Hamamelidaceae and Saxifragaceae are still not adequate. Another rDNA 18S sequences analysis (Hoot & Crane, 1996) indicates that *Cercidiphyllum* should be placed into the Hamamelidaceae. By increasing samples of the Hamamelidaceae and Saxifragaceae, the present analysis seems to favor the results of Chase *et al*. (1993) and Soltis *et al*. (1997) rather than the result of Hoot & Crane (1996). According to *rbc*L and 18S gene sequence analyses, the Cercidiphyllaceae is much closer to the Hamamelidaceae than to the Platanaceae, which is very different from the results of the cladistic analyses based on morphological characters.

In fact, more than one hundred years ago, Baillon proposed in 1871 that *Cercidiphyllum* might have affinity to the Hamamelidaceae, which was supported by Solereder in 1899 (cf. Endress, 1986). The pairwise genetic distance (PGD) values between *Cercidiphyllum* and any other genus of the Hamamelidaceae are in a range of 25～34(Table 3), which are in the range of genetic distance values among the subfamilies (23～36) of the Hamamelidaceae. The PGD values between *Cercidiphyllum* and *Daphniphyllum* is 37, while those between *Cercidiphyllum* and any other genera of the Saxifragaceae are in a range of 41～104. Therefore, both the Cercidiphyllaceae and Daphniphyllaceae are close to the Hamamelidaceae. The same result is also obtained in our preliminary ITS sequence analysis (unpublished).

In the Hamamelidaceae, *Fortunearia* and Hamamelis form a well supported clade. These two genera belong to the largest subfamily Hamamelidoideae, and they share a number of synapomophies such as 1-ovuled carpel, deciduous and pinnately veined leaves, moderately sized stipules, flowers with sepals and petals and basic chromosome number x = 12.

Prior to being erected as a separate family, *Daphniphyllum* had been placed into the Rhamnaceae by Blume in 1826, Meisner in 1836 and Endlicher in 1840(cf. Sutton, 1989), or into the Euphorbiaceae(Bentham, 1880). Stebbins(1974) treated it as a separate family. Hallier placed *Daphniphyllum* near the Hamamelidaceae in 1904(cf. Sutton, 1989), which was supported by the primitive wood structure (Carlquist, 1982), morphological characters of flower and fruit(Huang, 1965), pollen morphology (Bhatnagar & Garg, 1977), embryology (Mohana Rao, 1974), sieve-element plastids(Behnke, 1989, 1982, 1980) and chemical compounds (Giannasi, 1986). Our *rbc*L gene trees suggest that *Daphniphyllum* to be close to the Hamamelidaceae. Therefore, based on the *rbc*L gene sequences and some morphological characters we propose that both the Cercidiphyllaceae and Daphniphyllaceae be placed in the Hamamelidales.

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摘要 测定了悬铃木科和金缕梅科5个亚科的6个代表种的*rbc*L基因序列,对低等金缕梅类植物及新近提出的相关类群进行了分子系统发育分析,获得4个最简约树。简约树步长为893,其CI值和RI值分别为0.558和0.591。连香树科和交让木科与金缕梅科及虎耳草科关系较近。尽管增加了金缕梅科的取样密度,它们之间进一步的关系仍未得到分辨,结合它们的形态特征,连香树科和交让木科应置于金缕梅目内。*rbc*L基因树上反映出传统的"低等"金缕梅类成员领春木科与毛茛类植物聚在一起;悬铃木科与昆栏树科和水青树科关系较近,而与金缕梅科关系较远;杜仲科与低等金缕类的核心科——金缕梅科的关系似乎较除连香树科之外的其它低等金缕类成员近。低等金缕类植物由一些古老、孤立的科组成,且是多系的。

关键词 "低等"金缕梅类植物; 连香树科; 交让木科; 分子系统发育; *rbc*L基因